



# Origin, formation and environmental significance of *des*-A-arborenes in the sediments of an East African crater lake

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## ABSTRACT

Non-hopanoid pentacyclic triterpenoids occur widely in lake sediments, but their biological sources and diagenetic pathways are not fully resolved. We tentatively identified a number of *des*-A-arborene isomers occurring in relatively high abundance in the 25,000 year (25 kyr) sedimentary record of Lake Chala, a deep crater lake in tropical East Africa. The mono-, di- and tri-unsaturated *des*-A-arborenes are transformation products of isoarborinol/arborinone. These precursors have an aquatic source and are most likely biosynthesized by algae or aerobic bacteria in the epilimnion. The relatively depleted  $\delta^{13}\text{C}$  values (on average  $-32.3\text{‰} \pm 1.3\text{‰}$ ) of *des*-A-arbor-9(11)-ene are consistent with an aquatic source (algae or aerobic bacteria). In general, isoarborinol and its microbially induced transformation products are found in present and ancient tropical lacustrine settings (typically crater lakes) with permanently anoxic bottom waters and sediments. Based on molecular mechanics calculations of *des*-A-arborenes, it is clear that these transformation products are not in thermodynamic equilibrium, strongly indicating that their formation is microbially mediated. Subtle temporal and spatial differences in the microbial community might therefore not only dictate the variable relative contributions of different *des*-A-arborenes found in the sediments of Lake Chala over the last 25 kyr, but also explain the distribution of arborane derivatives in comparable crater-lake settings elsewhere.

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## 1. Introduction

Non-hopanoid pentacyclic triterpenoids preserved in lake and marine sediments are relatively well studied biomarkers and are predominantly used as a proxy for the terrestrial input of angiosperm higher plants (e.g., Rullkötter et al., 1982; Freeman et al., 1994; Sabel et al., 2005). The biological origin of sedimentary triterpenoids with an arborane carbon skeleton is, however, relatively unknown (e.g., Borrego et al., 1997; Volkman, 2005), so their use as biomarkers in paleoenvironmental reconstruction is limited. Arborane-type triterpenoids such as isoarborinol are known to occur in members of a few angiosperm families (Rutaceae: Vorbrüggen et al., 1963; Rubiaceae: Hui and Lam, 1965; Poaceae: Ohmoto et al., 1970; Euphorbiaceae: Hemmers et al., 1989), but

their general scarcity in higher plants seems to be at odds with the widespread occurrence and relative abundance of arborane-type triterpenoids found in lake sediments (e.g., Albrecht and Ourisson, 1969; Jaffé and Hausmann, 1995). Furthermore, aromatic isoarborinol derivatives have been found in sediments predating the evolution of angiosperm plants (Hauke et al., 1992a, 1992b, 1995). Alternative sources for arborane triterpenoid hydrocarbons, such as algae or aerobic bacteria, have also been proposed (Ourisson et al., 1982; Hauke et al., 1992b, 1995; Borrego et al., 1997; Volkman, 2005), but it has proven difficult to confirm their biosynthesis by these alternative sources. Only recently, the aerobic marine heterotrophic bacterium *Eudoraea adriatica* has been shown to produce arborane triterpenols (Banta et al., 2017).

During high-resolution stratigraphic analysis of *n*-alkanes (Sinninghe Damsté et al., 2011), *n*-alk-1-enes (van Bree et al., 2014) and *des*-A-lupane (van Bree et al., 2016) in the continuous and well-dated 25 kyr sedimentary record of Lake Chala, a deep equatorial crater lake on the border of Kenya and Tanzania, we also

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encountered a series of *des-A*-arborenes. *Des-A*-arborenes are diagenetic products of pentacyclic triterpenoids with an arborane skeleton such as isoarborinol and arborinone produced by microbial cleavage of the A-ring (Trendel et al., 1989; Lohmann et al., 1990). In this paper we report on the origin, transformation pathways and potential applications of the pentacyclic triterpenoid transformation products with an arborane skeleton in Lake Chala and compare our setting with other known lacustrine deposits containing arboranes. Molecular mechanics were applied to provide critical information regarding the relative stability of various (stereo)-isomers of *des-A*-arborenes, and the likeliness of diagenetic pathways of these compounds to more stable sedimentary biomarkers (cf. van Duin et al., 1997; van Duin and Sinninghe Damsté, 2003).

## 2. Material and methods

### 2.1. Study area

Lake Chala (3°19'S, 37°42'E; locally 'Challa', after a nearby village) is a small, deep and relatively unproductive tropical freshwater crater lake on the lower south-eastern slope of Mt. Kilimanjaro (Fig. 1). It is located on the border of Kenya and Tanzania, ~880 m above sea level, with a surface area of ~4.2 km<sup>2</sup>, a catchment area of ~3.4 km<sup>2</sup> and a maximum depth of around 90 m. The bi-annual passing of the Inter-Tropical Convergence Zone (ITCZ) results in moderate "long rains" (March–May) and heavy "short rains" (October–December), and consequently a long dry season (June–September). Subsurface groundwater inflow originating from rainfall on the mid-elevation slopes of Mt. Kilimanjaro is the main water source into the lake (Payne, 1970), although temporary discharge from a creek breaching the crater rim in the northwest can occur during very heavy rains (Buckles et al., 2014). Daily wind-driven water-column mixing is limited to 15–20 m year-round, while seasonal deep mixing (June–September) extends to 40–60 m. Consequently, the lower water column is permanently stratified. Due to the limited density stratification, complete mixing cannot be excluded, but its recurrence frequency is almost certainly decadal or longer, not inter-annual (Wolff et al., 2011, 2014).

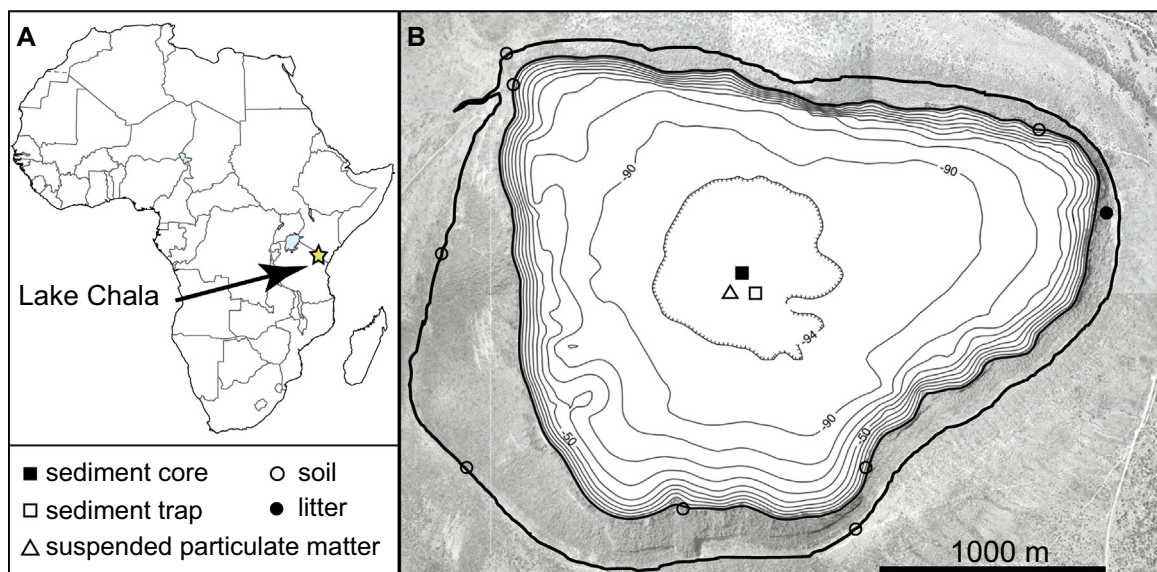
### 2.2. Study materials

We studied Lake Chala's finely laminated organic muds retrieved mid-lake (Fig. 1) in 2003 and 2005 (see Verschuren et al., 2009). The well-dated 20.82 m long master sequence covers the last 25 kyr of continuous offshore lacustrine sedimentation (see Blaauw et al., 2011). For this study, a total of 141 sediment samples with 4 cm thickness were extracted and processed for biomarker analysis, generally at ~200 year intervals, with a higher resolution of ~50 year intervals in the youngest 3.2 kyr, similar to previous studies (van Bree et al., 2014, 2016). In addition, we analyzed the compound-specific stable carbon isotopic composition of selected biomarkers in 14 sediment samples distributed throughout the sequence.

We also analyzed sediment trap, suspended particulate matter (SPM), soil and litter samples (Sinninghe Damsté et al., 2009; Buckles et al., 2014; van Bree et al., 2014, 2016). A sediment trap deployed at 35 m water depth in oxic water (~5 mg L<sup>-1</sup>) was retrieved at ca. 4 week intervals between November 2006 and December 2007 as described by Sinninghe Damsté et al. (2009). On 10–11 September 2006, a suite of SPM samples was obtained from throughout the water column, filtered and processed as described in van Bree et al. (2014 and 2016). Soil (*n* = 8) and litter (*n* = 5) samples were collected within the catchment area of Lake Chala in 2005 and 2012, respectively (Fig. 1). These were stored and processed as described by Sinninghe Damsté et al. (2009) and van Bree et al. (2016).

### 2.3. Lipid extraction

Lipid extraction has been performed in the context of previous studies (Sinninghe Damsté et al., 2011; van Bree et al., 2014, 2016). In short, the freeze-dried and powdered sediments were extracted with a Dionex™ Accelerated Solvent Extractor (ASE), using a dichloromethane (DCM)/methanol (9:1, v/v) mixture at high temperature (100 °C) and pressure (7.6 × 10<sup>6</sup> Pa). Subsequently the extracts were separated into an apolar and a polar fraction over an activated Al<sub>2</sub>O<sub>3</sub> column with hexane/DCM (9:1, v/v) and DCM/MeOH (1:1, v/v), respectively. A subset of the apolar fractions were



**Fig. 1.** Panel A shows the location of Lake Chala (3°19'S, 37°42'E) in East Africa, on the border of Kenya and Tanzania. Panel B shows lake bathymetry at 10 m intervals and locations of the suspended particulate matter (SPM) sampling (△), the sediment trap (□) and the 25 kyr sediment record (■), as well as catchment soil (○) and litter (●) collection. The outer bold line is the crater rim confining the catchment area, and the inner bold line the shoreline at the time of coring in 2005. Modified after Moernaut et al. (2010) and van Bree et al. (2014, 2016).

separated with a small Ag<sup>+</sup>-impregnated silica column into a saturated (hexane eluent) and an unsaturated hydrocarbon fraction (ethyl acetate eluent) prior to stable carbon isotopic composition analysis.

SPM and sediment trap samples were extracted previously (Sinninghe Damsté et al., 2009). Fluxes of apolar compounds (in mg m<sup>-2</sup> yr<sup>-1</sup>) in the water column were calculated using the concentration of each of these components relative to total particle flux measured in the sediment trap. Litter and soil samples were extracted ultrasonically with DCM/MeOH (2:1, v/v) as described by van Bree et al. (2016). In short, the extracts were methylated with diazomethane in diethyl ether and separated over an activated Al<sub>2</sub>O<sub>3</sub> column into apolar, ketone and polar fractions, using hexane/DCM (9:1, v/v), hexane/DCM (1:1, v/v) and DCM/MeOH (1:1, v/v) as eluents, respectively.

#### 2.4. Lipid identification and quantification

As described previously (Sinninghe Damsté et al., 2011; van Bree et al., 2014, 2016), the apolar fractions of sediments, SPM, sediment trap, soil and litter extracts were analyzed by gas chromatography (GC) and GC–mass spectrometry (GC–MS). A selection of polar fractions from samples of sediment, sediment trap and SPM were methylated, silylated and screened for functionalized triterpenoids.

GC was performed using a Hewlett-Packard (HP6890) instrument equipped with an on-column injector, a flame ionization detector (FID), a fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 CB (film thickness 0.12 μm), and helium as carrier gas. The samples were injected at 70 °C with an oven program of 20 °C min<sup>-1</sup> to 130 °C, 4 °C min<sup>-1</sup> to 320 °C, and held for 20 min. GC–MS was performed on a Finnigan Trace DSQ mass spectrometer operated at 70 eV with a mass range of *m/z* 40 to 800 and a cycle time of 1.7 s. The gas chromatograph was equipped with a fused silica capillary column as described above, with helium as carrier gas, and the same oven temperature program as for GC. Identification of the *des-A*-triterpenoids and other triterpenoid hydrocarbons is based on relative retention times, published mass spectra (including the NIST98 spectral library), and interpretation of observed fragmentation patterns. Quantification of compounds was performed by peak area integration of peaks in the FID chromatograms, including an internal standard. The concentration of apolar compounds are normalized to μg gTOC<sup>-1</sup>.

#### 2.5. Compound-specific carbon isotope analyses

We measured the compound specific δ<sup>13</sup>C signature of 20 unsaturated hydrocarbon fractions with an Agilent 6800 GC coupled to a ThermoFisher Delta V isotope ratio monitoring mass spectrometer. Samples were run in duplicate or triplicate, allowing an estimation of the standard deviation of the measurements. The isotope values were measured with reference to a calibrated external gas. Instrument performance was monitored daily by injections of a mixture of a C<sub>20</sub> and a C<sub>24</sub> perdeuterated *n*-alkane with known isotopic composition. The δ<sup>13</sup>C values are reported in standard delta notation against the Vienna Pee Dee Belemnite (VPDB).

#### 2.6. Molecular mechanics

We performed both quantum-chemistry-based Density Functional Theory (DFT) and ReaxFF classical-force-field simulations to calculate the relative stabilities of various stereo-isomeric configurations of the *des-A*-arboresenes. DFT calculations are deemed more accurate, but require more computational power than ReaxFF. The ReaxFF method has been well established over the last decade to describe a wide range of systems including catalysis,

combustion, pyrolysis, batteries, and other complex chemical systems (e.g., van Duin et al., 2001; Senftle et al., 2016). In the main text, we will only discuss the DFT results. Background, methodology and results from the ReaxFF simulations are available in the [Supplementary material](#), including a discussion on similarities and differences compared to DFT.

The DFT calculations were carried out with the [Jaguar 7.5 program](#) with the B3LYP (Becke, 1988; Lee et al., 1988) hybrid functional and the 6-311++G\*\* basis set. The mixture composition of various stereo-isomers at equilibrium was calculated using the following formula:

$$\%C_i = 100 \times \frac{\exp\left(\frac{\Delta G_i - \Delta G_l}{RT}\right)}{1 + \sum_{n=2}^{n_c} \exp\left(\frac{\Delta G_n - \Delta G_l}{RT}\right)}$$

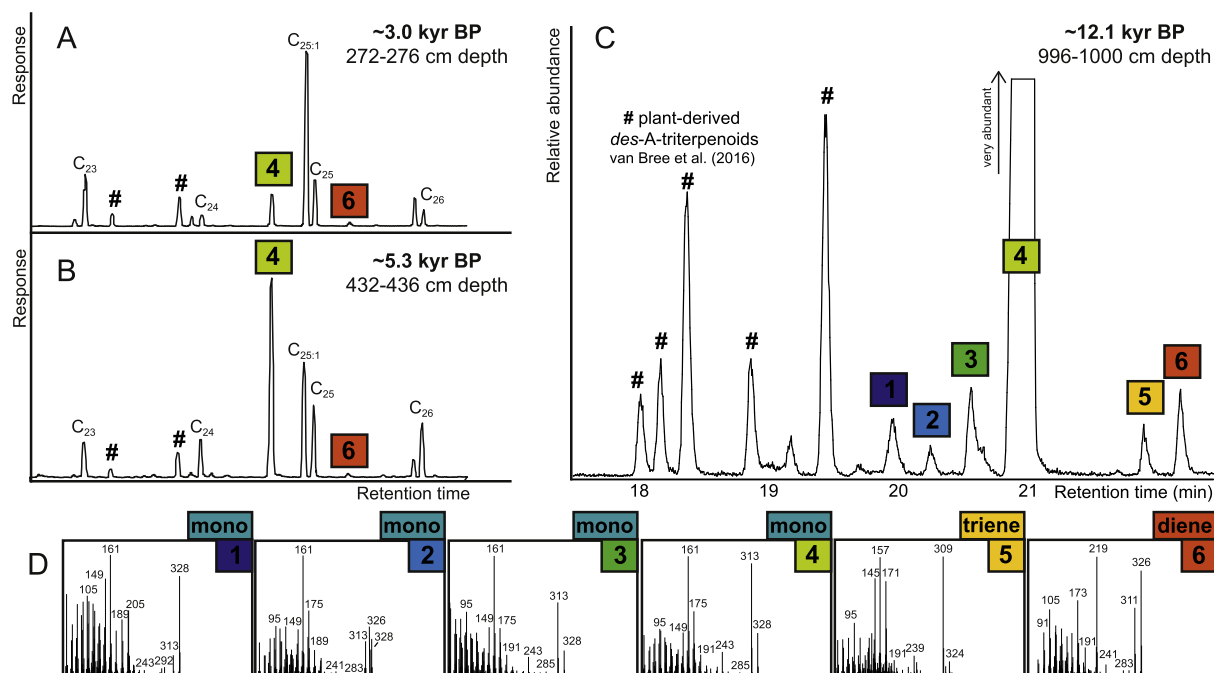
where Δ*G<sub>i</sub>* is the relative Gibbs free energy of isomer *i*, *n<sub>c</sub>* the number of compounds, *R* the universal gas constant, and *T* the temperature. In the equilibrium composition calculations at 298 K, the entropic contributions are approximated negligible, thus Gibbs free energies are replaced by the ground state energies.

### 3. Results

#### 3.1. The sedimentary record of *des-A*-triterpenoids in Lake Chala

Analysis of the apolar fractions of biomarker extracts from 141 well-dated sediment horizons covering the last 25 kyr revealed the presence of various biomarkers, including *n*-alkanes, *n*-alk-1-enes, phytadienes, di- and tri-aromatic triterpenoids, hopanoids, and *des-A*-triterpenoids with oleanane, ursane, lupane and arborane skeletons. The origin and paleoclimatic significance of some of these biomarkers have been discussed previously and include terrestrial long-chain *n*-alkanes (Sinninghe Damsté et al., 2011), aquatic C<sub>25:1</sub> and C<sub>27:1</sub> long-chain *n*-alk-1-enes (van Bree et al., 2014), and the higher plant-derived *des-A*-triterpenoids with oleanane, ursane and lupane skeletons (van Bree et al., 2016). Here we focus on the origin, transformation, distribution and potential paleoenvironmental application of the arborane-type *des-A*-triterpenoid hydrocarbons.

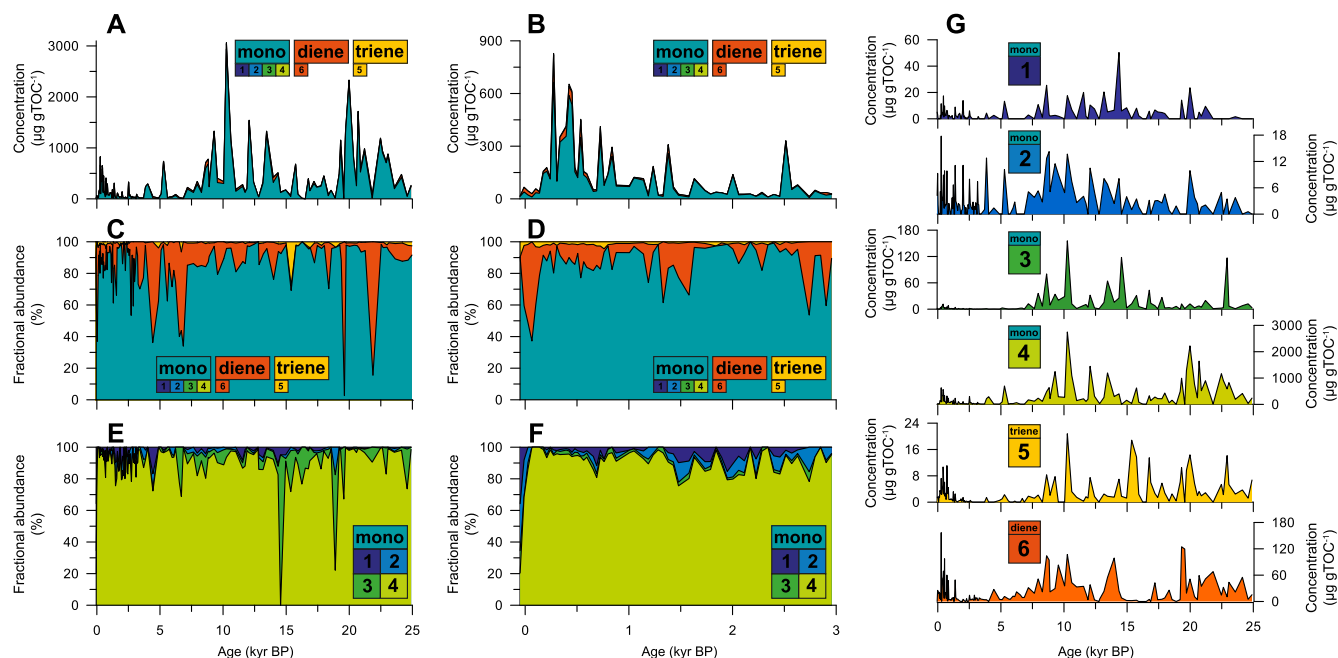
In the C<sub>23</sub> to C<sub>26</sub> *n*-alkane region of the chromatograms (Fig. 2A and B), six different peaks were identified as *des-A*-arboresenes with one to three double bonds, based on their relative retention times, published mass spectra, and fragmentation patterns. Fig. 2C shows a representative summed mass chromatogram (*m/z* 161 + 177 + 189 + 203 + 218 + 309) of a sediment horizon dated to 12.1 kyr BP, exemplifying their distribution. Compounds **1**, **2**, **3** and **4** (M<sup>+</sup> at *m/z* 328, base peak at *m/z* 161) reflect mass spectral fragmentation patterns similar to mono-unsaturated *des-A*-arboresenes (Fig. 2D). The presence of a fragment at *m/z* 326 in the mass spectrum of compound **2** indicates co-elution with an unknown *des-A*-arboradiene. The mass spectral fragmentation patterns of compounds **3** and **4** are similar to that of *des-A*-arbor-9(11)-ene as tentatively described by Jaffé and Hausmann (1995) and Jacob et al. (2007), possibly indicating the presence of two stereoisomers. Compound **5** (M<sup>+</sup> at *m/z* 324) is tentatively identified as *des-A*-arbor-5,7,9-triene (Hauke et al., 1992a). Compound **6** (M<sup>+</sup> at *m/z* 326) is tentatively identified as *des-A*-arbor-5(10),9(11)-diene (Jaffé and Hausmann, 1995). Some sedimentary horizons contain traces of other *des-A*-arboradienes, including two that coelute with a compound containing a mass fragment at *m/z* 329. Several other, non-identified *des-A*-triterpenoids occur infrequently or in trace amounts; these compounds are not further discussed. In addition, trace amounts of isoarborinol were found in polar fractions of suspended particulate matter (in the sample set discussed by van Bree et al., 2018) and in surface sediments.



**Fig. 2.** Distribution of *des-A*-triterpenoids in the Lake Chala sediment record. Panels A and B show partial GC-FID traces of the apolar fractions of hydrocarbon extract from representative sediment horizons at 272–276 cm and 432–436 cm depth, deposited ~3.0 and ~5.3 kyr BP, respectively. Panel C shows a summed mass chromatogram ( $m/z$  163 + 177 + 189 + 203 + 218 + 309) illustrating the distribution of *des-A*-triterpenoids from the apolar lipid fraction of a sample taken at 996–1000 cm depth, which is dated ~12.1 kyr BP; # indicates plant-derived *des-A*-triterpenoids (van Bree et al., 2016). Panel D shows the mass spectra of compounds **1** and **2** (mono/di-unsaturated *des-A*-triterpenes with an arborane skeleton), **3** and **4** (*des-A*-arbor-9(11)-ene), **5** (*des-A*-arbora-5,7,9-triene), and **6** (*des-A*-arbora-5(10),9(11)-diene); these identifications are tentative. Possible structures are shown in Fig. 5.

The distributions and abundances of these unsaturated *des-A*-arbores in the sedimentary record vary considerably over time. The summed record of the *des-A*-arbores (compounds **1**–**6**) shows three periods of high concentrations, namely 23.1–19.2 kyr BP, 14.0–8.3 kyr BP and around 0.4 kyr BP (Fig. 3A and D).

*Des-A*-arbores, and compound **4** in particular, are at times dominant in the apolar fraction of Lake Chala's lipid extracts. The overall average concentration is ~300  $\mu\text{g gTOC}^{-1}$  with peak values up to 3 mg  $\text{gTOC}^{-1}$  for the summed *des-A*-arbores. The relative proportion of mono-enes, dienes and trienes fluctuates over the past



**Fig. 3.** Distribution of *des-A*-arbores (in  $\mu\text{g gTOC}^{-1}$ ) in Lake Chala sediments over the past 25 kyr. Panel A shows the summed concentration of *des-A*-arbores (in  $\mu\text{g gTOC}^{-1}$ ) in Lake Chala sediments. Panel B shows the relative abundances, revealing a large proportion of monoenes (sum of **1**, **2**, **3** and **4**; green), and smaller proportions of the triene (**5**; yellow) and diene (**6**; orange). Panel C shows the fractional abundance of the mono-enes. Panels D, E, and F zoom in on these plots over the past 3 kyr, the section investigated at high temporal resolution. In panel G the *des-A*-arbores concentrations (in  $\mu\text{g gTOC}^{-1}$ ) of compounds **1**, **2**, **3**, **4**, **5** and **6** over the last 25 kyr are plotted separately. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



25 kyr (Fig. 3B). The mono-enes are dominated by compound **4**, but in few cases by compound **3** (Fig. 3C). The mono-enes are dominant in most samples (average 83%, median 91%). The fractional abundance of the diene (**6**) is highly variable and ranges between 0 and 100% (average 15%, median 8%). The triene (**5**) is typically present as a minor (<5%) component (average 1.8%, median 0.8%), but has peak occurrences of up to 35%, and is entirely absent in some intervals even though the sedimentary record has been sampled at very high resolution (e.g., between 2.7 and 3.7 kyr BP;  $n = 14$ ). When the dienes and trienes are relatively abundant, the overall concentration of unsaturated *des*-A-arborenes is always relatively low (Fig. 3).

The stable carbon isotopic composition of *des*-A-arborene compound **4** was determined successfully in 14 out of 20 sediment horizons (Fig. 4), and ranges from  $-29.8\text{‰}$  to  $-33.8\text{‰}$  ( $-32.3\text{‰} \pm 1.3\text{‰}$  on average). Reliable determination of the stable carbon isotopic composition of other *des*-A-arborenes was unsuccessful due to co-elution or low abundances.

### 3.2. (*Des*-A)-triterpenoids in soil and litter surrounding the lake, in settling particles and suspended particulate matter

No *des*-A-triterpenoids were identified in the hydrocarbon extracts from soils and litter, and no functionalized arborane-type triterpenoids were detected in other fractions. Van Bree et al. (2016) reported the presence of functionalized triterpenoids with oleanane, ursane and lupane skeletons in the soil and litter, but arborane or fernane-type triterpenoids were not encountered. In the extracts of particulate matter recovered with the sediment trap, only low concentrations of *des*-A-urs-13(18)-ene and *des*-A-lupane were detected, next to traces of functionalized triterpenoids such as  $\alpha$ -amyrinol. Hydrocarbon concentrations in the SPM were too low for identification of specific triterpenoid compounds.

### 3.3. Molecular mechanics: DFT simulation

DFT molecular mechanics calculations were performed to calculate the stabilities of the isomers of mono/di/tri-unsaturated *des*-A-arborenes (Table 1; Fig. 5). If isomerization of double bonds via relatively stable tertiary carbocations (de Leeuw et al., 1989) occurs, five double bond isomers, i.e.  $\Delta^{5(10)}$ ,  $\Delta^7$ ,  $\Delta^8$ ,  $\Delta^9$ , and  $\Delta^{9(11)}$ , may result from the original  $\Delta^{9(11)}$  *des*-A-arborene. Isomerization

of the  $\Delta^{9(11)}$ -isomer via the tertiary carbocation at C-9 can produce  $\Delta^8$ - and  $\Delta^9$ -isomers. The  $\Delta^8$ -isomer can give rise to  $\Delta^7$ -isomers via the tertiary carbocation at C-8, whereas the  $\Delta^9$ -isomer can evolve into the  $\Delta^{5(10)}$ -isomer via the tertiary carbocation at C-10. Chiral centers in *des*-A-arbora-monoenes occur at C-8, C-9, and C-10, and, hence, stereoisomers may occur as well. As the double bond isomers of *des*-A-arbora-monoenes can have 2 or 4 stereo-isomers each, there are 16 possible configurations (see Fig. 5 for roman numeral codes). DFT calculations indicate that generally, the  $\Delta^{9(11)}$ -isomers are most stable (**IIIa-b**), followed by  $\Delta^7$  (**IIIg**),  $\Delta^8$  (**IIIe-f**) and  $\Delta^9$  (**IIIk**). More specifically, DFT molecular mechanics calculations indicate that  $\Delta^{9(11)}$ -8 $\beta$ H,10 $\beta$ Me (**IIIa**) is the most stable *des*-A-arbora-monoene (Table 1, Fig. 5). The DFT calculations predict stabilities for  $\Delta^{9(11)}$ -8 $\alpha$ H,10 $\alpha$ Me (**IIIb**),  $\Delta^8$ -10 $\alpha$ Me (**IIIe**),  $\Delta^8$ -10 $\beta$ Me (**IIIf**),  $\Delta^7$ -9 $\alpha$ H,10 $\alpha$ Me (**IIIg**), and  $\Delta^9$ -8 $\beta$ H (**IIIk**) that are less stable but are within 2 kcal/mol, compared to that of  $\Delta^{9(11)}$ -8 $\beta$ H,10 $\beta$ Me (**IIIa**). Hence, if thermodynamic equilibrium exists for the *des*-A-arborenes, these compounds should be present in significant amounts (Table 1).

Isomerization of *des*-A-arboradienes (**IIa** to **IId**) can only occur via relatively stable allylic tertiary carbocations (de Leeuw et al., 1989). Hence, only four possible *des*-A-arboradienes may be formed starting from the  $\Delta^{9(11)}$ -isomer:  $\Delta^{5(10),9(11)}$ -8 $\beta$ H (**IIa**),  $\Delta^{5(10),9(11)}$ -8 $\alpha$ H (**IIb**),  $\Delta^{5(10),8}$  (**IIc**), and  $\Delta^{7,9}$  (**IId**; Fig. 5). The DFT calculations indicate that  $\Delta^{5(10),9(11)}$ -8 $\beta$ H (**IIa**) is the most stable isomer and would dominate the mixture ( $\sim 99.9\%$ ) if at equilibrium.

We also calculated the inter-conversion energetics of the most stable monoene (**IIIa**), diene (**IIa**) and B-ring aromatized  $\Delta^{5,7,9}$  *des*-A-arboratriene (**IV**), considering molecular hydrogen as reference for hydrogenation and dehydrogenation reactions. DFT calculations predict an exothermic conversion process of diene **IIa** to monoene **IIIa** ( $\Delta E_1 = -14.10$  kcal/mol), and endothermic conversions for both diene **IIa** to triene **IV** ( $\Delta E_2 = 1.13$  kcal mol $^{-1}$ ), and monoene **IIIa** to triene **IV** ( $\Delta E_3 = 15.50$  kcal mol $^{-1}$ ).

## 4. Discussion

### 4.1. Arborane vs fernane precursors

One important issue regarding *des*-A-arborenes is the possibility that they may have been formed by transformation of fernane-type triterpenoids, since *des*-A-ferrenes are enantiomers of *des*-A-arborenes (Jaffé and Hausmann, 1995). Arborane and fernane skeletons (Fig. 5: **1a**–**1d**) differ only in stereochemistry due to chair and boat conformations of the B-ring when they are synthesized (Ohmoto et al., 1970). Therefore, its precursor skeleton is no longer identifiable when aromatized or when the A-ring is removed. Fernane-type triterpenoids have been reported from different biological sources including plants, lichens and bacteria (e.g., Ohmoto et al., 1970; Bottari et al., 1972; Howard et al., 1984; Hemmers et al., 1989; Shiojima and Ageta, 1990; González et al., 1991; Hauke et al., 1992b; Paull et al., 1998; Maier et al., 2009). Although a fernane-type origin cannot be ruled out completely, it is highly unlikely in Lake Chala, firstly due to the presence of trace amounts of isoarborinol and absence of functionalized fernenes in the modern water column and surface sediments; and secondly due to the similarity of Lake Chala with other lacustrine environments from where large amounts of arborane-triterpenoids have been reported (Section 4.2). Therefore, we are most likely dealing with compounds derived from functionalized arboranes, more specifically isoarborinol and/or arborinone.

### 4.2. Comparison with other lacustrine records

Isoarborinol and its derivatives are often found in lake settings similar to Lake Chala. Sometimes large quantities of isoarborinol/

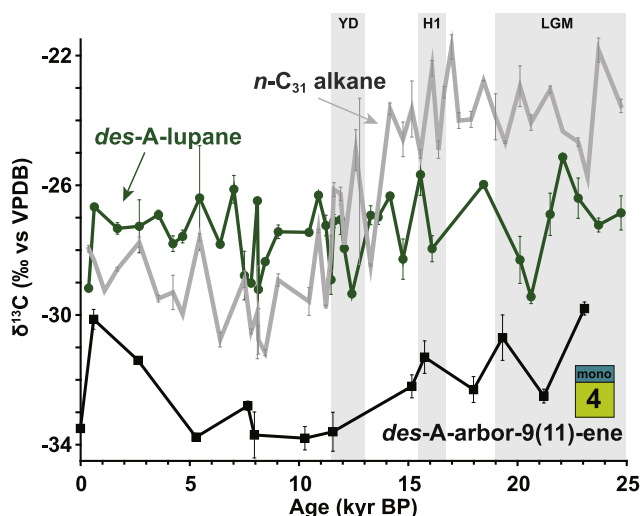


Fig. 4. The stable carbon isotopic composition ( $\delta^{13}\text{C}$ , in  $\text{‰}$  vs VPDB) of *des*-A-arborene compound **4** vs time, in comparison with the records for the  $\text{C}_3$  plant-derived *des*-A-lupane (van Bree et al., 2016) and the  $n\text{-C}_{31}$  alkane plant waxes (Sinninghe Damsté et al., 2011).

**Table 1**  
DFT molecular calculation results of double bond and stereoisomers of *des*-A-arborene, -diene and -triene.  $\Delta E_{\text{DFT}}$  marked with an \* are significantly present in equilibrium compositions calculated at 298 K, and their equilibrium composition (%) is calculated for mono-enes and dienes separately.  $\Delta E$  was calculated compared to compounds **IIc** and **IIle**, marked with an #.

Structure	Double bond position	Stereoisomer configuration	DFT (kcal/mol)	$\Delta E_{\text{DFT}}$ (kcal/mol)	Equilibrium composition (%)
<b><i>des</i>-A-arbor-diene</b>					
<b>IIa</b>	$\Delta^{5(10),9(11)}$	8 $\beta$ H	−588380.49	−4.29*	99.9
<b>IIb</b>	$\Delta^{5(10),9(11)}$	8 $\alpha$ H	−588376.94	−0.74	–
<b>IIc</b>	$\Delta^{5(10),8}$	–	−588376.80	# 0.00	–
<b>IId</b>	$\Delta^{7,9}$	–	−588335.71	40.48	–
<b><i>des</i>-A-arbor-monoene</b>					
<b>IIIa</b>	$\Delta^{9(11)}$	8 $\beta$ H,10 $\beta$ Me	−589145.22	−0.84*	57.5
<b>IIIb</b>	$\Delta^{9(11)}$	8 $\alpha$ H,10 $\alpha$ Me	−589144.48	−0.09*	16.4
<b>IIIc</b>	$\Delta^{9(11)}$	8 $\beta$ H,10 $\alpha$ Me	−589141.99	2.40	0.2
<b>IIId</b>	$\Delta^{9(11)}$	8 $\alpha$ H,10 $\beta$ Me	−589140.16	4.23	0.0
<b>IIIe</b>	$\Delta^8$	10 $\alpha$ Me	−589144.39	# 0.00*	14.0
<b>IIIf</b>	$\Delta^8$	10 $\beta$ Me	−589143.70	0.69*	4.4
<b>IIIg</b>	$\Delta^7$	9 $\alpha$ H,10 $\alpha$ Me	−589143.88	0.51*	6.0
<b>IIIh</b>	$\Delta^7$	9 $\alpha$ H,10 $\beta$ Me	−589140.93	3.46	0.0
<b>IIIi</b>	$\Delta^7$	9 $\beta$ H,10 $\beta$ Me	−589139.49	4.90	0.0
<b>IIIj</b>	$\Delta^7$	9 $\beta$ H,10 $\alpha$ Me	−589138.26	6.13	0.0
<b>IIIk</b>	$\Delta^9$	8 $\beta$ H	−589143.02	1.36*	1.4
<b>IIIl</b>	$\Delta^9$	8 $\alpha$ H	−589138.22	6.17	0.0
<b>IIIm</b>	$\Delta^{5(10)}$	8 $\beta$ H,9 $\alpha$ H	−589140.74	3.65	0.0
<b>IIIn</b>	$\Delta^{5(10)}$	8 $\alpha$ H,9 $\alpha$ H	−589138.55	5.83	0.0
<b>IIIo</b>	$\Delta^{5(10)}$	8 $\beta$ H,9 $\beta$ H	−589135.09	9.29	0.0
<b>IIIp</b>	$\Delta^{5(10)}$	8 $\alpha$ H,9 $\beta$ H	−589131.80	12.58	0.0
<b><i>des</i>-A-arbor-triene</b>					
<b>IV</b>	$\Delta^{5,7,9}$	–	−587639.18	–	–

arborinone are found in small (sub-) tropical freshwater lakes with a permanently anoxic hypolimnion (Table 2). This includes modern lakes such as Lake Valencia (Venezuela; Jaffé and Hausmann, 1995) and Lake Albano (Italy; Hanisch et al., 2003), but also paleolake deposits such as the Eocene Lake Messel ( $\sim 47.8 \pm 0.2$  Ma; Albrecht and Ourisson, 1969; Kimble et al., 1974; Hauke et al., 1992a), Eckfeld Maar ( $\sim 44.3$  Ma; Sabel et al., 2005), the Buxwiller shales (Arpino et al., 1971; Hauke et al., 1995), the Ménat shales (Hauke et al., 1992a, 1995), and the Miocene Clarkia deposits (Logan and Eglinton, 1994; Huang et al., 1995). Isoarborinol/arborinone is usually found in highly productive (eutrophic to hypereutrophic) lakes, while Lake Chala is oligotrophic. Typically, isoarborinol is found in tropical or warm and humid climates, but it has also been detected in the Pleistocene sediments of the Arctic Lake El'gygytyn (D'Anjou et al., 2013). Lake Valencia and paleolake Messel are the most comparable to Lake Chala. Lake Valencia is much larger than Lake Chala, but its sedimentary lipid content is very similar, not only with respect to arborane-triterpenoid derivatives (isoarborinol, *des*-A-arbor-9(11)-ene, *des*-A-arbor-5(10),9(11)-diene), but also for example with respect to the abundance of algal long-chain *n*-alk-1-enes (van Bree et al., 2014). The depositional environment of Lake Chala is similar to that of the Eocene Lake Messel, which contains *des*-A-arbor-5,7,9-triene **IV** and high abundances of isoarborinol/arborinone (Albrecht and Ourisson, 1969; Hauke et al., 1992a).

#### 4.3. Microbial origin of isoarborinol/arborinone

There are various potential biological sources of the *des*-A-arborene precursors isoarborinol and arborinone. Traditionally they are attributed to a terrestrial higher plant source (e.g., Albrecht and Ourisson, 1969; Ohmoto et al., 1970; Vliex et al., 1994; Jacob et al., 2005), but they have also been assigned to phytoplankton and bacteria (Ourisson et al., 1982; Hauke et al., 1992b, 1995; Jaffé and Hausmann, 1995; Volkman, 2005). Oxidosqualene cyclases (OSCs), the enzymes responsible for the biosynthesis of C3-oxygenated cyclic triterpenoids, are predominantly occurring

in the eukaryotic domain, but a limited number of bacterial OSCs has also been identified (Banta et al., 2017). The only confirmed bacterial production of arborane triterpenols is by the aerobic marine heterotrophic bacterium *Eudoraea adriatica* (Banta et al., 2017). However, metagenomic sequences from both marine and lacustrine environments indicated the presence of the bacterial OSC responsible for the biosynthesis of arborane triterpenols, suggesting the possibility of other bacterial sources for these types of triterpenols.

##### 4.3.1. Stable carbon isotopic composition

One way of differentiating between terrestrial and aquatic source of lipid biomarkers is the carbon isotopic composition of specific lipids (Fig. 4). In Lake Chala,  $\delta^{13}\text{C}$  values of compound **4** range from  $-29.8\text{‰}$  to  $-33.8\text{‰}$  ( $-32.3\text{‰} \pm 1.3\text{‰}$  on average). No isotopic fractionation is expected to occur during transformation of triterpenoids (Freeman et al., 1994), therefore these  $\delta^{13}\text{C}$  values are thought to reflect the  $\delta^{13}\text{C}$  signature of the parent compound and can be used to distinguish between alternative plant or microbial sources. The relative  $^{13}\text{C}$  depletion found here is comparable to that reported for arborane compounds in other lake settings. In a study of Eocene shales (Hauke et al., 1992a), *des*-A-arbor-5,7,9-triene (**IV**) has a  $\delta^{13}\text{C}$  value of  $-28.7\text{‰}$ , contrasting with significantly less depleted  $\delta^{13}\text{C}$  values ( $-23\text{‰}$  to  $-25\text{‰}$ ) of lupane or ursane-type triterpenoids. Such a clear offset between the  $\delta^{13}\text{C}$  values of terrestrial triterpenoid derivatives and arborane derivatives is also seen in Lake Chala (Fig. 4), where compound **4** is significantly more depleted in  $^{13}\text{C}$  ( $-29.8\text{‰}$  to  $-33.8\text{‰}$ ;  $-32.3\text{‰} \pm 1.3\text{‰}$  on average) than the plant-derived *des*-A-lupanes ( $-25.1\text{‰}$  to  $-29.4\text{‰}$ ;  $-27.4\text{‰} \pm 1.1\text{‰}$  on average; van Bree et al., 2016). Isoarborinol is reported with  $\delta^{13}\text{C}$  values of  $-28.0\text{‰}$  to  $-28.4\text{‰}$  in Miocene Lake Clarkia (Logan and Eglinton, 1994; Huang et al., 1995), and  $-24.7\text{‰}$  to  $-27.9\text{‰}$  in Lake Valencia (Xu and Jaffé, 2008). The significant  $^{13}\text{C}$  depletion of compound **4** in Lake Chala sediments indicates that here an aquatic source is much more likely than a terrestrial higher plant source of arborane-type triterpenoids.

POTENTIAL PRECURSORS	<div data-bbox="268 193 536 417"> <b>Ia</b>              (iso)arborinol            arborinol: 3<math>\alpha</math>   isoarborinol: 3<math>\beta</math> </div> <div data-bbox="571 193 839 395"> <b>Ib</b>              arborinone         </div> <div data-bbox="874 193 1142 395"> <b>Ic</b>              ferneol         </div> <div data-bbox="1177 193 1445 395"> <b>Id</b>              fernenone         </div>
DIENES	<div data-bbox="268 438 552 640"> <b>IIa</b>              8<math>\beta</math>H-<i>des</i>-A-arbora-5(10),9(11)-diene  <math>\Delta E_{DFT} = -4.29</math> kcal/mol         </div> <div data-bbox="571 438 855 640"> <b>IIb</b>              8<math>\alpha</math>H-<i>des</i>-A-arbora-5(10),9(11)-diene  <math>\Delta E_{DFT} = -0.74</math> kcal/mol         </div> <div data-bbox="874 438 1158 640"> <b>IIc</b>    <i>des</i>-A-arbora-5(10),8-diene  <math>\Delta E_{DFT} = 0</math> kcal/mol *         </div> <div data-bbox="1177 438 1461 640"> <b>IId</b>    <i>des</i>-A-arbora-7,9-diene  <math>\Delta E_{DFT} = 40.48</math> kcal/mol         </div>
MONO-ENES	<div data-bbox="268 661 552 874"> <b>IIIa</b>              8<math>\beta</math>H,10<math>\beta</math>Me-<i>des</i>-A-arbora-9(11)-ene  <math>\Delta E_{DFT} = -0.84</math> kcal/mol         </div> <div data-bbox="571 661 855 874"> <b>IIIb</b>              8<math>\alpha</math>H,10<math>\alpha</math>Me-<i>des</i>-A-arbora-9(11)-ene  <math>\Delta E_{DFT} = -0.09</math> kcal/mol         </div> <div data-bbox="874 661 1158 874"> <b>IIIc</b>              8<math>\beta</math>H,10<math>\alpha</math>Me-<i>des</i>-A-arbora-9(11)-ene  <math>\Delta E_{DFT} = 2.40</math> kcal/mol         </div> <div data-bbox="1177 661 1461 874"> <b>IIId</b>              8<math>\alpha</math>H,10<math>\beta</math>Me-<i>des</i>-A-arbora-9(11)-ene  <math>\Delta E_{DFT} = 4.23</math> kcal/mol         </div> <div data-bbox="268 895 552 1108"> <b>IIIe</b>              10<math>\alpha</math>Me-<i>des</i>-A-arbora-8-ene  <math>\Delta E_{DFT} = 0.00</math> kcal/mol *         </div> <div data-bbox="571 895 855 1108"> <b>IIIf</b>              10<math>\beta</math>Me-<i>des</i>-A-arbora-8-ene  <math>\Delta E_{DFT} = 0.69</math> kcal/mol         </div> <div data-bbox="874 895 1158 1108"> <b>IIIg</b>              9<math>\alpha</math>H,10<math>\alpha</math>Me-<i>des</i>-A-arbora-7-ene  <math>\Delta E_{DFT} = 0.51</math> kcal/mol         </div> <div data-bbox="1177 895 1461 1108"> <b>IIIh</b>              9<math>\alpha</math>H,10<math>\beta</math>Me-<i>des</i>-A-arbora-7-ene  <math>\Delta E_{DFT} = 3.46</math> kcal/mol         </div> <div data-bbox="268 1129 552 1342"> <b>IIIi</b>              9<math>\beta</math>H,10<math>\beta</math>Me-<i>des</i>-A-arbora-7-ene  <math>\Delta E_{DFT} = 4.90</math> kcal/mol         </div> <div data-bbox="571 1129 855 1342"> <b>IIIj</b>              9<math>\beta</math>H,10<math>\alpha</math>Me-<i>des</i>-A-arbora-7-ene  <math>\Delta E_{DFT} = 6.13</math> kcal/mol         </div> <div data-bbox="874 1129 1158 1342"> <b>IIIk</b>              8<math>\beta</math>H-<i>des</i>-A-arbora-9-ene  <math>\Delta E_{DFT} = 1.36</math> kcal/mol         </div> <div data-bbox="1177 1129 1461 1342"> <b>IIIl</b>              8<math>\alpha</math>H-<i>des</i>-A-arbora-9-ene  <math>\Delta E_{DFT} = 6.17</math> kcal/mol         </div> <div data-bbox="268 1364 552 1566"> <b>IIIm</b>              8<math>\beta</math>H,9<math>\alpha</math>H-<i>des</i>-A-arbora-5(10)-ene  <math>\Delta E_{DFT} = 3.65</math> kcal/mol         </div> <div data-bbox="571 1364 855 1566"> <b>III n</b>              8<math>\alpha</math>H,9<math>\alpha</math>H-<i>des</i>-A-arbora-5(10)-ene  <math>\Delta E_{DFT} = 5.83</math> kcal/mol         </div> <div data-bbox="874 1364 1158 1566"> <b>IIIo</b>              8<math>\beta</math>H,9<math>\beta</math>H-<i>des</i>-A-arbora-5(10)-ene  <math>\Delta E_{DFT} = 9.29</math> kcal/mol         </div> <div data-bbox="1177 1364 1461 1566"> <b>IIIp</b>              8<math>\alpha</math>H,9<math>\beta</math>H-<i>des</i>-A-arbora-5(10)-ene  <math>\Delta E_{DFT} = 12.58</math> kcal/mol         </div>
TRIENES	<div data-bbox="268 1576 647 1736"> <b>IV</b>    <i>des</i>-A-arbora-5,7,9-triene         </div> <div data-bbox="874 1576 1174 1661">           Present in significant amount in calculated equilibrium mixture         </div> <div data-bbox="855 1693 1238 1736">           * reference compound for <math>\Delta E_{DFT}</math> calculations         </div>

**Fig. 5.** Molecular structures and configuration of potential precursors (**Ia–Id**), *des*-A-arbora-dienes (**IIa–IId**), *des*-A-arborenes (**IIIa–IIIp**) and *des*-A-arbora-triene (**IV**). Also indicated are the  $\Delta E_{DFT}$  values calculated for the dienes and mono-enes relative to **IIc** and **IIIe**, respectively. Isomers accentuated in blue are present in significant amounts in the calculated equilibrium mixtures. The reference compounds for  $\Delta E_{DFT}$  calculations (**IIc** and **IIIe**) are indicated with an asterisk (\*). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

#### 4.3.2. Distribution patterns of *des*-A-arborenes

Besides the depleted  $\delta^{13}\text{C}$  values, also the distribution of *des*-A-arborenes in Lake Chala sediments is different from those of known higher plant-derived biomarkers. Indeed, the concentration of

*des*-A-arborenes in the sediment is often higher than those of bona fide terrestrial higher plant biomarkers such as *n*-alkanes and the plant-derived *des*-A-triterpenoids. The concentration of compound **4** is not correlated with that of *des*-A-lupane, *des*-A-ursenes and

**Table 2**  
Characteristics of Lake Chala compared to other (paleo-)lakes with a sedimentary record of various arborane-type triterpenoid compounds.

Lake	Location	(paleo-) climate	Salinity	Age	Lake characteristics	Arborene-compounds reported	Interpretation precursor origins	Ref.
Chala	Kenya/ Tanzania	Tropical	Fresh	0–25 kyr BP (Quaternary)	Volcanic crater lake with an area of 4.5 km <sup>2</sup> . Average depth 81 m, maximum depth ~90 m. Oligotrophic. Permanently anoxic hypolimnion.	<i>des</i> -A-arbor-9(11)-enes; <i>des</i> -A-arbora-5(10),9(11)-diene; <i>des</i> -A-arbora-5,7,9-triene; isoarborinol (trace)	Microbial	<b>This study</b>
Valencia	Venezuela	Tropical	Fresh	0–13 kyr BP (Quaternary)	Origin lake due to faulting and damming of the Valencia river. Hypereutrophic lake with an area of 350 km <sup>2</sup> . Average depth 19 m, maximum depth 40 m. Permanently anoxic hypolimnion.	<i>des</i> -A-arbor-9(11)-ene; <i>des</i> -A-arbora-5(10),9(11)-diene; isoarborinol; arborinone	Microbial	<b>A B</b>
Messel oil shales	Germany	Tropical	Fresh	47.8 ± 0.2 Ma (Eocene)	Volcanic crater lake with an area of 1.7 km <sup>2</sup> . Depth between 10 s and 100 s of m. Hypereutrophic with a permanently anoxic hypolimnion.	isoarborinol; arborinone; <i>des</i> -A-arbora-5,7,9-triene; 24,25-dinorarbora-1,3,5(10),9(11)-tetraene; 24,25-dinorarbora-1,3,5,7,9-pentaene	Microbial	<b>C</b>
Eckfeld Maar	Germany	Tropical	Fresh	44.3 ± 0.4 Ma (Eocene)	Small crater lake (<1 km <sup>2</sup> ). Depth of 160–210 m. Eutrophic, with a permanently anoxic hypolimnion.	arborinone, <i>des</i> -A-arbora-9(11)-ene, <i>des</i> -A-arbora-5,7,9-triene, 24,25-dinorarbora-1,3,5,7,9,11-hexaene, 24,25-dinorarbora-3,5,7,9-tetraene, 24,25-dinorarbora-1,3,5,7,9-pentaene, 24,25-dinorarbora-5,7,9-triene	Microbial	<b>D</b>
Albano	Italy	Mediterranean	fresh	Holocene (Quaternary)	Volcanic crater lake with an area of 6 km <sup>2</sup> . Average depth of 77 m, 175 m maximum. Trophic state is meso- to eutrophic, with a permanently anoxic hypolimnion.	isoarborinol; arborinone	Phytoplankton, other microbial origin possible	<b>E</b>
Clarkia lacustrine deposits	Idaho, USA	Tropical	Fresh	15.4–16 Ma (Miocene)	Drainage basin dammed by flood basalts. Deeper than 12 m. Trophic state is meso- to eutrophic, with a permanently anoxic hypolimnion.	isoarborinol; arborinone	Terrestrial (original studies), but microbial possible	<b>F G</b>
Kupfer- schiefer	Germany		Lagoonal	Permian	Permanently anoxic hypolimnion.	<i>des</i> -A-arbor-8-ene; B-ring aromatized ferenes; <i>des</i> -A-arbora-5,7,9-triene; 24,25-dinorarbora-1,3,5(10),9(11)-tetraene; 24,25-dinorarbora-1,3,5,7,9-pentaene	Pre-angiosperm deposits, therefore interpreted as gymnosperm terrestrial origin (H; I), but possible microbial source. B-ring aromatized compounds are fernane-type (optical rotation), while the triene is from isoarborinol (NMR)	<b>H I J K</b>
Bouxwiller shales	France	Tropical	Fresh	Eocene		isoarborinol; arborinone		<b>K L</b>
Caçó	Brazil	Tropical	Fresh	0–20 kyr BP (Quaternary)	Lake with an area <2.5 km <sup>2</sup> , and less than 12 m deep. Oligotrophic, no permanently anoxic hypolimnion.	arbor-9(11)-en-3β-ol methyl ester	Terrestrial	<b>M N</b>
Orbagnoux Basin	France		Lagoonal	Kimmeridgian (Late Jurassic)		<i>des</i> -A-arbora-5,7,9-triene	Microbial origin in pre-angiosperm deposits. Derived from ferenes (O) or derived from isoarborinol (K)	<b>K O</b>



Table 2 (continued)

Lake	Location	(paleo-) climate	Salinity	Age	Lake characteristics	Arborene-compounds reported	Interpretation precursor origins	Ref.
Puertollano oil shale	Spain		Fresh	~300 Ma (Carboniferous; Late Stephanian)		<i>des-A-arbora-5,7,9-triene</i> ; <i>des-E-D-C-friedo-25-norhopa-5,7,9-triene</i> ; 25-norarbora(ferna)-triene; 24,25-dinorarbora(ferna)-1,3,5,7,9-pentaene; <i>iso-25-norarbora(ferna)-1,3,5,7,9-pentaene</i>	Microbial (bacterial) origin in pre-angiosperm deposits. Uncertain from arbora- or fernane-type triterpenoids.	C
El'gygygyn	Russia	Arctic	Fresh	275–475 kyr BP (Quaternary)	Impact crater lake with an area of 110 km <sup>2</sup> and a depth of 175 m. Oligotrophic, no permanently anoxic hypolimnion.	isoarborinol	Terrestrial interpretation (P); microbial or phytoplankton origin not discussed but possible.	P
Kinneret	Israel		Fresh	Holocene (Quaternary)	Lake with an area of 168 km <sup>2</sup> , and a maximum depth of 42 m. Holomictic (no permanently anoxic hypolimnion), but with anoxic sediments.	isoarborinol; arborinone	Bacterial	Q

A: Jaffé and Hausmann (1995), B: Xu and Jaffé (2008), C: Borrego et al. (1997), D: Sabel et al. (2005), E: Hanisch et al. (2003), F: Logan and Eglinton (1994), G: Huang et al. (1995), H: Schwark and Püttmann (1990), I: Bechtel and Püttmann (1992), J: Hauke et al. (1992a), K: Hauke et al. (1995), L: Arpino et al. (1971), M: Jacob et al. (2005), N: Jacob et al. (2007), O: Hauke et al. (1992b), P: D'Anjou et al. (2013) and Q: Robinson et al. (1986).

*des-A-oleanenes* ( $R^2 = 0.01$ ,  $0.02$ , and  $0.08$ , respectively; van Bree et al., 2016). Although the *des-A-arborenes* are at times dominant in apolar sediment extracts from Lake Chala, their potential precursors (i.e., triterpenoid ketones, alcohols, methyl ethers, acetates and acids with an arborane skeleton) were not found in the soil and litter of the Lake Chala area, and only trace amounts of isoarborinol were detected in selected sediment samples. In conclusion, a land-derived origin of the *des-A-arborenes* in Lake Chala is much less likely than an aquatic, microbial origin.

#### 4.3.3. Comparison with similar geochemical settings

Not only in Lake Chala, but also in many small and permanently stratified tropical lakes (Table 2) an aquatic microbial origin is much more likely than a terrestrial one. Ourisson et al. (1982) already predicted an aerobic bacterial source for isoarborinol, as this compound has been found in many different sediments and is relatively rare in plants. Hauke et al. (1992a, 1992b) proposed that sedimentary arborane-type triterpenoids originate from aerobic bacteria or algae, based on their stable carbon isotopic composition and the requirement of molecular oxygen for their biosynthesis. A bacterial origin was also proposed by Jaffé and Hausmann (1995) in their study of the hypereutrophic Lake Valencia. Analysis of SPM from Lake Valencia showed the absence of arborane-type triterpenoids (Jaffé et al., 1996), whereas these hydrocarbons occurred abundantly in the sediment. Isoarborinol derivatives from the Eocene Eckfeld Maar in Germany are also thought to have a bacterial origin and coincide with the occurrence of a diatom-rich community (Sabel et al., 2005), which is of interest since diatoms are also the dominant group of phytoplankton in Lake Chala. The occurrence of isoarborinol derivatives in other lacustrine deposits predating the development of angiosperm higher plants in the Cretaceous (Hauke et al., 1995; Borrego et al., 1997) is interpreted as a strong argument for a non-higher plant source of the precursor compounds.

Although most studies conclude that a microbial aquatic origin of arborane triterpenoids is most probable, in some settings a terrestrial origin is possible. For example, in Lake Caçó the arborane triterpenoids are of terrestrial origin (Jacob et al., 2007). Although Lake Caçó is a small, tropical, oligotrophic, freshwater lake like Chala, it is relatively shallow and has no permanent anoxic hypolimnion and is in that respect different to the small crater lakes where arborane triterpenoids are most likely of microbial aquatic origin (Table 2). In other lakes and shales, the interpretation of arborane derivatives as of terrestrial or aquatic origin is more ambiguous. For example, when there is known input of fernane-type triterpenoids or when the depositional setting is different (e.g., the Kupferschiefer and Orbagnoux sites; see Table 2), or when the possibility of a microbial aquatic origin has not been studied. For example, *des-A-arbor-8,9-enes* from the Permian Kupferschiefer sediments have been interpreted as terrestrial, due to the large co-occurring quantities of bisaccate conifer pollen in the sedimentary record during anoxic freshwater depositional conditions (Bechtel and Püttmann, 1992). Using optical rotation techniques, Hauke et al. (1995) demonstrated that the arborenes/fernanes IV in these Permian sediments where in fact isoarborinol-derived transformation products. Therefore, co-occurrence of pollen and arborenes by no means excludes a microbial aquatic origin, but this was not discussed in the original study.

Based on interpretation of isoarborinol  $\delta^{13}\text{C}$  values, a terrestrial origin has also been suggested for arborane compounds in the Miocene Clarkia deposits (Logan and Eglinton, 1994; Huang et al., 1995). However, isoarborinol from Clarkia is more depleted in  $^{13}\text{C}$  ( $-28.0\text{‰}$  to  $-28.4\text{‰}$ ) than are terrestrial *des-A-lupane* ( $-25.1\text{‰}$ ) and oleanane-triterpenoids ( $-26.2\text{‰}$ ; Logan and Eglinton, 1994; Huang et al., 1995). Due to their biosynthetic pathway, triterpenoids in plants are 5–6‰ enriched in  $^{13}\text{C}$  compared to

straight-chain compounds (Diefendorf et al., 2012). When Clarkia  $\delta^{13}\text{C}$  values of isoarborinol ( $-28.0\text{‰}$  to  $-28.4\text{‰}$ ) are compared to those of *n*-alkanes ( $-27.4\text{‰}$  to  $-32.8\text{‰}$ ) taking into account this triterpenoid enrichment, it is not evident that the isoarborinol is of terrestrial origin. An aquatic source of isoarborinol in Lake Clarkia is therefore more likely.

#### 4.3.4. Aerobic production of *des*-A-arborenes precursors

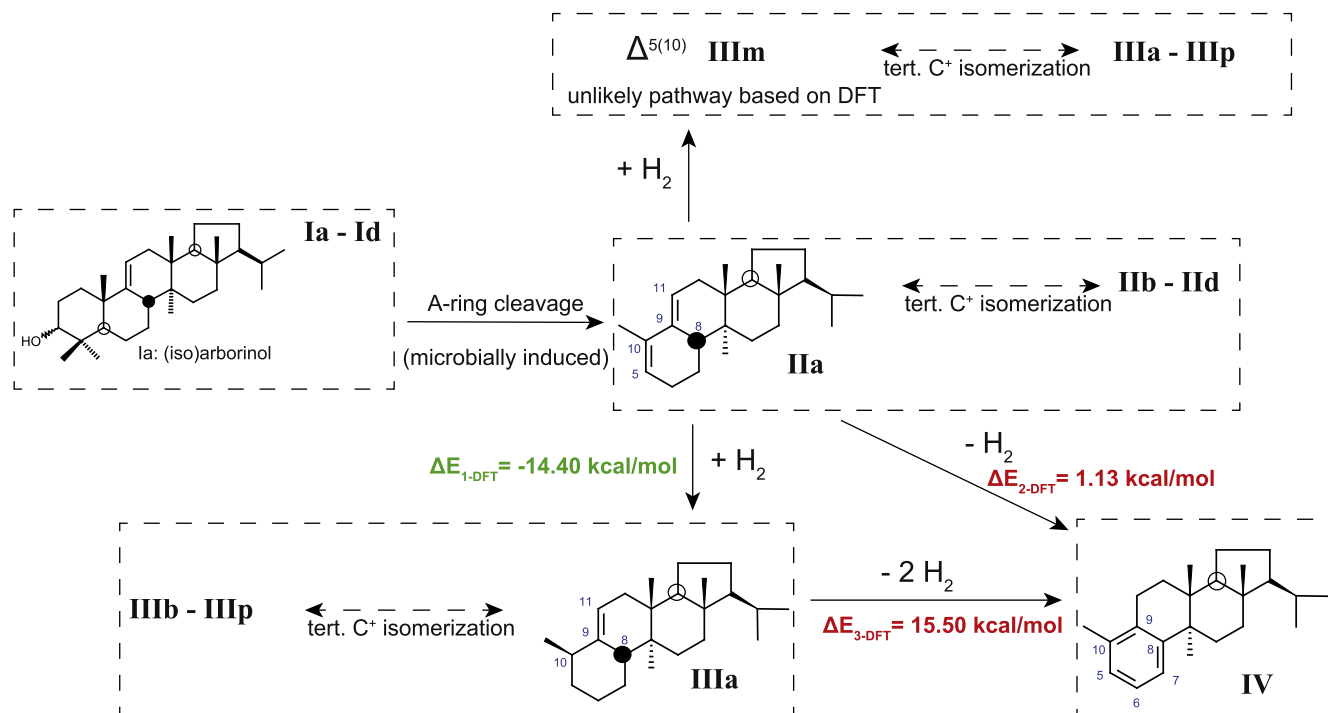
The mere presence of ring-A degraded arborene-type triterpenoids in sediments indicates that their functionalized precursors must be produced by aerobic organisms in a (sub)oxic environment, because *des*-A-triterpenoids can only be formed when there is an oxygenated function present at C-3 (Lohmann et al., 1990). Triterpenoids with an oxygenated function at C-3 are formed by OSCs, which require oxidosqualene as the substrate. The biosynthesis of oxidosqualene requires molecular oxygen (Ourisson et al., 1982; Summons et al., 2006; Fischer and Pearson, 2007). This constrains the depth of production of the *des*-A-arborenes precursor(s) to the upper portion of the water column in Lake Chala, since the water column below 45–55 m is anoxic throughout the year. Oxygen penetration in the water column varies seasonally (Wolff et al., 2011, 2014; Buckles et al., 2014; van Bree et al., 2018), and during some stratified periods, anoxic conditions extend upwards to ~15 m depth. However, production of arborane triterpenoids in the modern-day water column of Lake Chala seems to be low. Isoarborinol is only present in trace amounts in settling particles and SPM, and *des*-A-arborenes were not detected at all. This makes the identification of a microbial production zone more challenging, although it is reasonably certain they are produced in the oxic or suboxic water column due to the aforementioned C-3 oxygenation. As the extent of the epilimnion and chemocline depth is seasonally variable, we do expect seasonal changes in the microbial production of isoarborinol, which may in turn be reflected in the variable concentration of arborane compounds in the sediment record (Fig. 3; see Section 4.5).

#### 4.4. Transformation pathways of isoarborinol: abiotic diagenesis vs microbial transformations

A suite of transformation products of isoarborinol/arborinone occurs in the sedimentary record of Lake Chala, including mono-unsaturated (1–4), di-unsaturated (5) and tri-unsaturated (mono-aromatic) (6) *des*-A-arborenes. One of the remaining questions is how these transformations from functionalized arborane triterpenoids to the *des*-A compounds take place, and if this is an abiotic or microbially mediated process. Molecular mechanics calculations can help solve this question. If abiotic transformations occur, such as the loss of ring A and possible isomerization through tertiary carbocations, one may expect that the mixture of *des*-A-arborenes is either in thermodynamic equilibrium or approaches it with depth.

##### 4.4.1. Microbial A-ring removal: diene formation

*Des*-A-arboradienes are probably the first product formed after A-ring removal. The process of A-ring removal is thought to be mediated by microbial activity under anoxic or reducing conditions (Lohmann et al., 1990; Jaffé and Hausmann, 1995; Jacob et al., 2007; Huang et al., 2008) and may yield four possible isomers of *des*-A-arboradienes: **IIa** to **IId** (Fig. 5), if we assume that double bond isomerization can take place through allyl-stabilized tertiary carbocations after loss of ring A. In Lake Chala sediments we detected only one diene in high abundance (compound **6**) and only minor traces of three other dienes in some of the sedimentary horizons. Compound **6** is, therefore, likely 8 $\beta$ H-*des*-A-arbora-5(10),9(11)-diene **IIa**, which is the most likely microbial product of (iso)arborinol and/or arborinone after A-ring loss and by far the most stable diene isomer based on molecular mechanics calculations (Table 1). Hence, we cannot conclude that **IIa** or **IIb** is the dominant *des*-A-arbora-diene produced after the loss of ring A, or if this dominant diene results from other dienes upon double bond isomerizations through tertiary carbocations.



**Fig. 6.** Schematic diagenetic pathway of (iso)arborinol. For clarification, only **Ia** (iso)arborinol is depicted, but the pathways are valid for all possible parent compounds **Ia-Id**. Hydrogenation of the 9(11) double bond is unlikely based on DFT calculations, but is depicted for completeness. Hydrogenation of **IIa** to **IIIa** is exothermic (green values), and the dehydrogenation of **IIa** and **IIIa** to **IV** is endothermic (red values). Tertiary C<sup>+</sup> isomerization is indicated where possible. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

#### 4.4.2. Mono-unsaturated *des-A*-arborenes: abiotic isomerisation vs microbially induced variation

*Des-A*-arbor-monoenes are formed by hydrogenation of *des-A*-arboradienes. As the  $\Delta^{5(10)}$  double bond hydrogenation (from **IIa** → **IIIa**) is exothermic (Fig. 6), this process is likely to happen spontaneously. This could explain the dominance of compound **4** in Chala sediments (Fig. 3C), which most likely has the **IIIa** ( $8\alpha\text{H}, 10\alpha\text{Me}-\Delta^{9(11)}$ ) configuration. In the sedimentary record, we observe substantial variation in the distributions of mono-unsaturated *des-A*-arborenes but no distinct trend with time (Fig. 3C and F). When we compare the sedimentary distribution of these isomers with their modeled distribution at thermodynamic equilibrium (Table 1), we see that the *des-A*-arbor-monoene isomers present in Lake Chala sediments are not in thermodynamic equilibrium, nor is there a trend towards thermodynamic equilibrium over time. This also indicates that even over a time span of 25 kyr, there is no significant abiotic transformation between the *des-A*-arborene isomers. This implies that the four different monounsaturated *des-A*-arborenes are more likely formed microbially, either directly after the loss of ring A or after microbial hydrogenation, and that the isomerization of double bonds via tertiary carbocations can be excluded as a transformation process. This implies further that spatial or temporal variations within the microbial community could be reflected in the different monoene distributions observed.

The  $\Delta^{9(11)}$  arbor-monoene has four possible stereoisomers:  $8\alpha\text{H}, 10\alpha\text{Me}$  (**IIIa**),  $8\alpha\text{H}, 10\beta\text{Me}$  (**IIIb**),  $8\beta\text{H}, 10\alpha\text{Me}$  (**IIIc**), and  $8\beta\text{H}, 10\beta\text{Me}$  (**IIId**). Two *des-A*-arbor-monoenes (compounds **3** and **4**) in Lake Chala sediments have mass spectra similar to *des-A*-arbor-monoenes published previously (Jaffé and Hausmann, 1995; Jacob et al., 2007). Double bond positions in arborane-type compounds are however difficult to differentiate based on mass-spectrometric fragmentation patterns alone, as they are similar for isomers of various pentacyclic triterpenoids and their diagenetic derivatives (cf. Shiojima et al., 1992; Lavrieux et al., 2011). Therefore, mass spectrometry alone will not be able to distinguish all four stereoisomers. The order of elution of the various *des-A*-arborene isomers is also unknown. Since there is no thermodynamic equilibrium observed, it is not possible to use molecular mechanics calculations to determine the individual stereochemical isomers of the  $\Delta^{9(11)}$ -ene isomers.

In other lacustrine settings, only *des-A*-arborenes with a  $\Delta^{9(11)}$  double bond have been described. In oil shales of the Duaringa Basin, Boreham et al. (1994) tentatively identified two *des-A*-arbor-9(11)-ene isomers with identical mass spectra but different retention times.  $10\alpha\text{Me}-des-A$ -arbor-9(11)-ene was tentatively identified in sediments of Lake Valencia (Jaffé and Hausmann, 1995) and Lake Caçó (Jacob et al., 2007). The identification of *des-A*-arborenes in all studies is tentative, and the stereochemistry at C-10 is either not discussed (Boreham et al., 1994) or inferred to be  $10\alpha\text{Me}$  (Jaffé and Hausmann, 1995; Jacob et al., 2007), although  $10\beta\text{Me}$  is the most stable isomer based on molecular mechanics calculations (Table 1). It can be expected that the most abundant compound **4** has the most stable configuration, which is  $8\beta\text{H}, 10\beta\text{Me}-des-A$ -arbor-9(11)-ene (**IIIa**), in contrast to **IIIc** ( $8\beta\text{H}, 10\alpha\text{Me}$ ) that was postulated by Jaffé and Hausmann (1995). Further studies, based on synthesized *des-A*-arborenes, are required to elucidate the stereochemistry of these compounds.

#### 4.4.3. Microbially induced *des-A*-arbor-triene formation

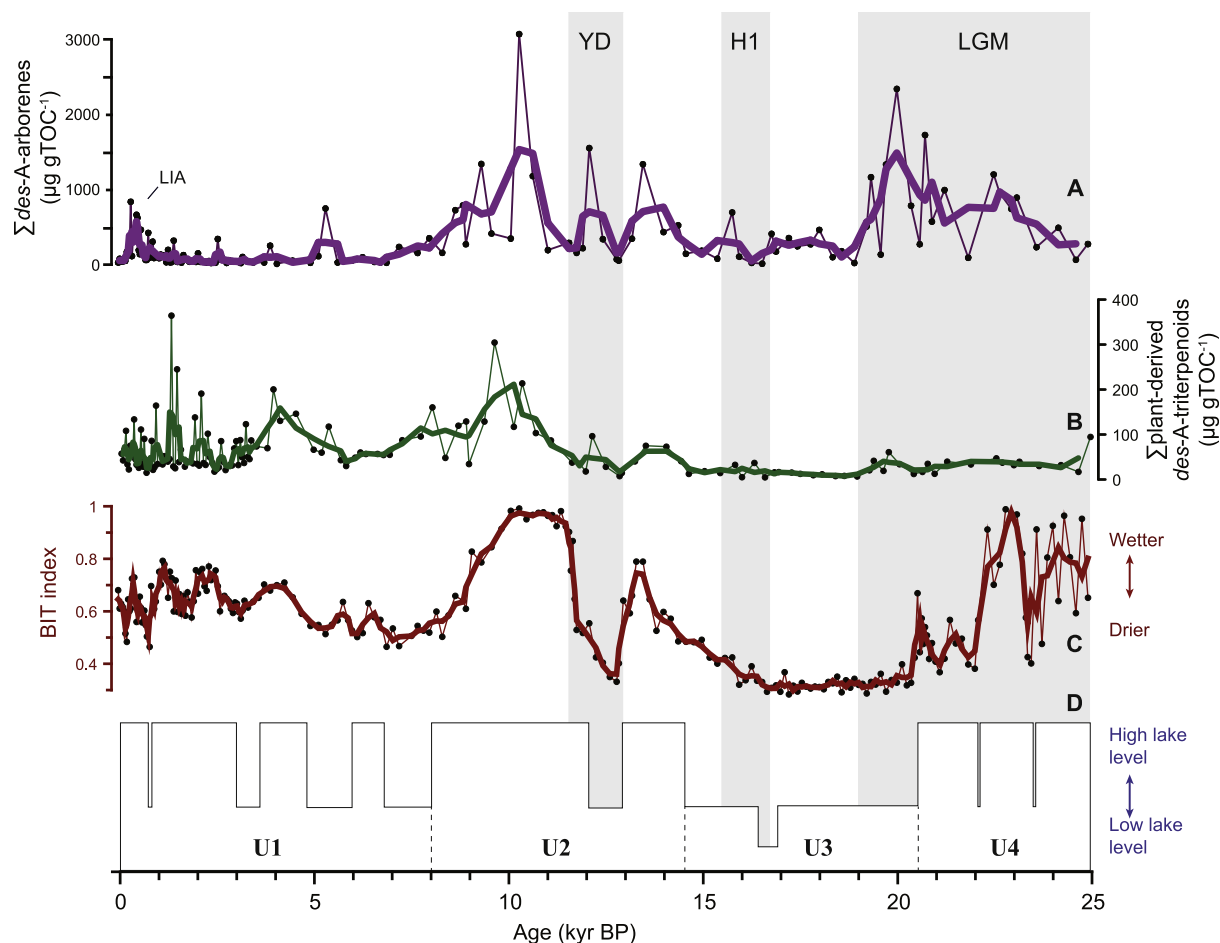
The mono-aromatic *des-A*-arborene **IV** occurs throughout the sedimentary record of Lake Chala (compound **5**, Fig. 3G). The transformation of dienes **II** and mono-enes **III** to the B-ring aromatized compound **IV** are strongly endothermic (Fig. 6), and although **IV** features an aromatic ring, and is therefore expected to be more stable than non-aromatic *des-A*-arborenes, molecular mechanics

calculations indicate it is not. The strongly endothermic transformation must thus involve enzymatic and/or inorganic catalytic processes. The idea that micro-organisms can induce molecular aromatization is not new. For example, Wakeham et al. (1980) and Lohmann et al. (1990) stress the importance of microorganisms in the degradation of triterpenoid precursors to polycyclic aromatic hydrocarbons within sediments. In Lake Chala, **IV** is already present in the profundal surface sediments. This whole transformation process from A-ring removal to B-ring aromatization, therefore, must take place relatively rapidly, within 40 years. This is much faster than observed in Lake Valencia sediments (Jaffé and Hausmann, 1995), where only small amounts of the B-ring aromatized *des-A*-arborene were detected at 45 cm depth, indicating that it took ~180 years to form small amounts of this compound. This difference could be due to different microbial communities in the water column and sediments.

#### 4.5. Application in paleoenvironmental reconstruction

As the transformation of isoarborinol and arborinone to the different *des-A*-arborenes depends on enzymatic action, it is likely that the microbial community is influenced by subtle spatial variation in the water column environment and thereby the structures and relative abundances of *des-A*-arborenes in the sediment. These differences in microbial communities will be most pronounced between different lake systems, but also within one system the microbial communities will differ seasonally and along a depth gradient. Although these microbial changes are likely to occur on short spatial and temporal scales, sediments can record these changes cumulatively in the form of long-term trends, and hence examining absolute and relative changes in sedimentary *des-A*-arborene degradation products through time provide insight in some physical aspects of Lake Chala. As only low (trace) amounts of isoarborinol are found in Lake Chala at present, it is reasonable to assume that the vast majority of triterpenoids originally present have been subjected to microbial transformation. Therefore, the accumulation of *des-A*-arborenes in the sediment record can be used as proxy for the production rates of isoarborinol/arborinone in the water column. These parent compounds are most likely synthesized by aerobic bacteria residing just above the chemocline of the lake (Section 4.3).

To elucidate possible paleoclimatological and paleoenvironmental controls on the concentration of *des-A*-arborenes in Lake Chala sediments, this record is compared with two independent proxy records (Fig. 7) of climatic moisture balance (the precipitation/evaporation ratio), namely the seismic-reflection record of lake level fluctuation (Moernaut et al., 2010) and the branched isoprenoid tetraether (BIT) index of past rainfall variations (Verschuren et al., 2009; Buckles et al., 2016). The concentration of *des-A*-arborenes is somewhat higher during inferred wetter periods, and lower during inferred drier periods (Fig. 7). While concrete evidence for a mechanistic link explaining this correlation is not provided by this study, we can infer that *des-A*-arborene production must have been higher during times of more pronounced water column stratification and/or higher precipitation than today. Variations over time between the different *des-A*-arborene compounds could indicate changes in the activity of different microbial assemblages producing and degrading isoarborinol or arborinone. It would be interesting to compare the proliferation of microbial communities in different lake systems where *des-A*-arborenes and their precursors have been found, as this could be informative to understand the subtleties in microbial transformation patterns of isoarborinol/arborinone. Future metagenomic studies of the modern lake system of Lake Chala and its sediments may reveal the connection between microbial communities and isoarborinol production and its microbial transformations.



**Fig. 7.** Summed concentration (in  $\mu\text{g gTOC}^{-1}$ ) of *des-A*-arbores in Lake Chala sediments [A], compared to the plant-derived *des-A*-triterpenoid record ( $\mu\text{g gTOC}^{-1}$ ) [B], the branched isoprenoid tetraether (BIT) index record (Verschuren et al., 2009) [C], and the seismic-reflection record of high- and lowstands (Moernaut et al., 2010) [D] over the past 25 kyr. Shaded areas represent the Last Glacial Maximum (LGM; 26.5–19 kyr BP), Heinrich event H1 (16.8–15.4 kyr BP) and the Younger Dryas period (YD; 13–11.5 kyr BP). The Little Ice Age is also marked (LIA; 1270–1750 CE). Points connected by a thin line are individual data, while thicker lines represent a three-point moving average.

## 5. Conclusions

The sedimentary record of Lake Chala contains a variety of transformation products of pentacyclic triterpenoids with an arborane skeleton. The functionalized triterpenoids are synthesized by an unverified microbial source in the water column, most likely algae or aerobic bacteria. After production in the (sub-)oxic water column, microbially mediated A-ring loss of isoarborinol/arborinone occurs in the suboxic/anoxic water column, producing  $8\beta\text{H}$ -*des-A*-arbor-5(10),9(11)-diene, which is hydrogenated forming predominantly  $8\beta\text{H}$ -10 $\beta\text{Me}$ -*des-A*-arbor-9(11)-ene. The process of aromatization from mono- and di-unsaturated *des-A*-arbores to *des-A*-arbor-5,7,9-triene is endothermic, and therefore most likely microbially mediated. Although their precise biological source remains unknown, *des-A*-arbores are specific biomarkers with high preservation potential in the anoxic sediments of Lake Chala, and in the anoxic sediments of similar small, tropical crater lakes with a permanently anoxic hypolimnion. The varying proportion of *des-A*-arbores in these systems could be a reflection of subtle differences in the physical parameters of the water column along the depth gradient, resulting in changing microbial communities and activity. Differences in arborane-type triterpenoids and their distribution patterns between different lakes, both extant and in the geological past, are likely due to different microbial communities producing and transforming isoarborinol and/or arborinone and *des-A*-arbores with one, two and three double bonds.

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## Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.orggeochem.2018.09.001>.

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